CHRONIC TOXICITY STUDIES ON FOOD COLOURS

PART II. OBSERVATIONS ON THE TOXICITY OF FD&C GREEN NO. 2 (LIGHT GREEN SF YELLOWISH), FD&C ORANGE NO. 2 (ORANGE SS) AND FD&C RED NO. 32 (OIL RED XO) IN RATS

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THIS paper describes further studies on the chronic toxicity of food colours. In Part I of this series some chronic effects of Oil Yellow AB and OB in rats were reported[1]. It has previously been reported by other workers that some colours belonging to the azo and triphenylmethane classes caused tumours to develop in animals after subcutaneous injections[2,3]. As some of these colours are being used in food it was thought worthwhile to examine them for chronic oral effects in rats. The effects of the oral administration of FD&C Green No. 2, FD&C Orange No. 2 and FD&C Red No. 32 on growth, food consumption, food efficiency, blood hæmoglobin, and on the pathology of a number of organs are presented in this paper.

METHODS

The methods employed were similar to those reported for the two yellow colours[1]. The food colours were incorporated in the laboratory diet in the following concentrations: FD&C Green No. 2, 0·03, 1·5 and 3·0 per cent; FD&C Orange No. 2 and FD&C Red No. 32, 0·03, 0·75 and 1·5 per cent. The rats were approximately five to six weeks of age at the start of the experiment. The animals were kept in groups of two to a cage and were given free access to their respective diets and water. Their body weight and food consumption were recorded weekly. For more accurate evaluation of food consumption it would have been preferable to place only one rat in a cage, but this was not possible. Post-mortem examinations were made where possible on rats which died on test. All surviving rats were killed at the end of the experiments and post-mortem examinations were made. Many of the organs were weighed and prepared for histological examination. As a result of an unfortunate accident at the end of the fourth week to the male rats on a dietary concentration of 1·5 per cent. FD&C Green No. 2, data on these rats are not included.

RESULTS AND DISCUSSION

The Effect on Growth Rate, Food Consumption and Food Efficiency

Growth, food consumption and food efficiency curves for the groups receiving the various dietary concentrations of the three colours are shown in Figures 1 and 2. Growth rate, food consumption and food

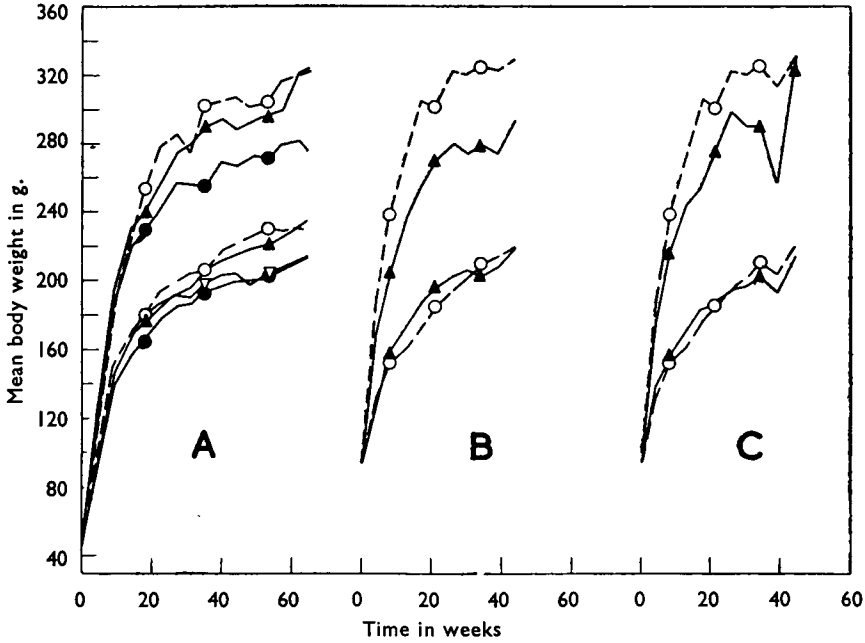

FIG. 1. Growth rate curves for control rats and those receiving the colours. Lower curves are for female rats and upper curves for male rats.

A. FD&C Green No. 2: B. FD&C Orange No. 2: FD&C Red No. 32

○ control; ▲ 0·03 per cent.; ▽ 1·5 per cent.; ● 3·0 per cent.

efficiency were not significantly affected by any of the three colours at the 0·03 per cent dietary level. However, for FD&C Red No. 32 and FD&C Orange No. 2 at the higher concentrations of 0·75 and 1·5 per cent. of the diet, growth rate, food consumption and food efficiency were noticeably affected and all rats in these two groups died before completion of the experiment. For FD&C Green No. 2, two dietary levels, 0·03 and 1·5 per cent. had no effect on growth rate, food consumption or food efficiency, but the 3·0 per cent. level noticeably affected growth rate. This may have been partly due to the amount of food consumed, which was somewhat reduced on this dietary amount. Daily doses of 200 mg./kg. and 400 mg./kg. of FD&C Red No. 32 and FD&C Orange No. 2 for 20 weeks to rats affected growth, food consumption and food efficiency, as shown in Tables I and IV.

The Effect of Mortality

At the end of the test period (65 weeks) the mortality of rats on FD&C Green No. 2 ranged from 52 to 68 per cent. for the respective groups, as shown in Table II. The control group mortality was 68 per cent. The mortality for the respective groups on dietary concentrations of FD&C Green No. 2 was not significantly different from the control. For the other two colours there was 100 per cent. mortality at the 0·75 and

TABLE I

SUMMARY OF DATA ON MORTALITY, FOOD CONSUMPTION AND FOOD EFFICIENCY WHEN FOOD COLOURS WERE GIVEN BY STOMACH TUBE FOR 20 WEEKS

Dose	Sex	No. rats on test	Mortality	Food consumption g./rat/day	Food efficiency g. gain/g. food consumed × 100
Control	M	10	0	13·2	5·2
	F	10	0	11·1	4·7
200 mg./kg./orally/daily .. FD&C Red No. 2 ..	M	10	6	12·5	2·9
	F	10	0	12·7	3·1
200 mg./kg./orally/daily .. FD&C Orange No. 2 ..	M	10	6	13·9	3·3
	F	10	2	10·3	4·5
400 mg./kg./orally/daily .. FD&C Red No. 32 ..	M	10	5	12·1	1·7
	F	10	3	10·1	1·8
400 mg./kg./orally/daily .. FD&C Orange No. 2 ..	M	10	6	10·3	1·9
	F	10	6	10·0	0·6

1·5 per cent. levels by the time the experiment was ended as shown in Table III. By the end of 20 weeks all the rats on the 1·5 per cent. level of FD&C Red No. 32 had died, and at the end of 40 weeks all the rats on the 0·75 per cent. level had died. It was not possible to make autopsies on all the animals which died during the experiment, but the tissues and organs of many of those dying on the 0·75 and 1·5 per cent. dosage were stained with the colours. The kidneys were soft, dark and swollen. The spleen was enlarged and dark in colour. The picture was one of acute toxæmia.

TABLE II

CUMULATIVE NUMBER OF DEATHS

Concentration of colour in diet	Sex	No. Rats on test	Time in weeks on test														
			1	3	5	10	15	20	25	30	35	40	45	50	55	60	65
FD&C Green No. 2																	
Control	M	25	1	6	7	8	10	11	12	13	14	14	14	14	14	16	17
	F	25	0	1	1	2	3	3	3	6	6	7	8	8	8	8	10
0·03 per cent.	M	25	0	0	0	1	1	2	4	8	8	8	8	9	11	13	14
	F	25	0	0	0	0	1	4	4	5	6	7	9	11	12	13	13
1·5 per cent.	F	25	0	0	0	0	1	1	2	2	2	3	5	7	13	13	14
3·0 per cent.	M	25	0	0	2	3	3	3	3	4	10	10	11	11	12	13	16
	F	25	0	0	0	2	2	3	4	6	8	8	9	10	10	14	14

The Effect on Organ Weights

Organs of surviving rats were weighed at the termination of the test. The mean weights (in mg./g. of body weight) are shown in Table IV. The mean weights of a number of organs deviated significantly from those of corresponding controls. Heart, liver, spleen, kidneys and testes were the organs chiefly affected. In very few instances where increases or decreases in organ weights occurred was it possible to demonstrate pathological changes; the changes demonstrated in the testes are an exception. In a number of cases the organ weights were about the

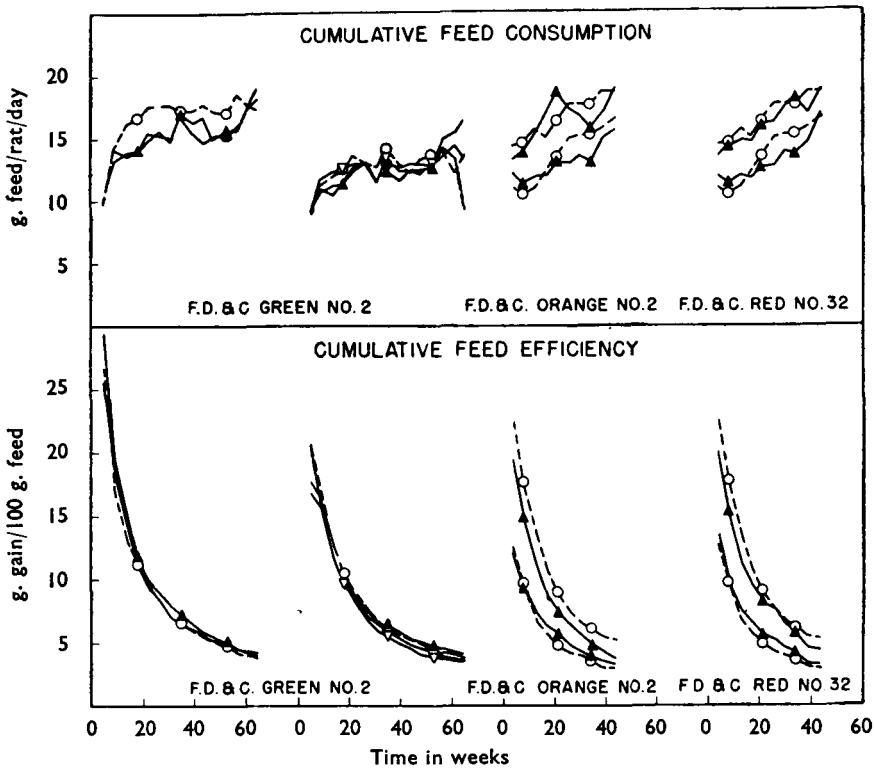

FIG. 2. Food consumption and food efficiency curves for control and test rats, male and female. For FD&C Green No. 2, values for males are shown on the left. For the other two colours, the upper curves represent male rats and the lower curves female rats.

○ Control; ▲ 0·03 per cent.; ▽ 1·5 per cent.; ● 3·0 per cent.

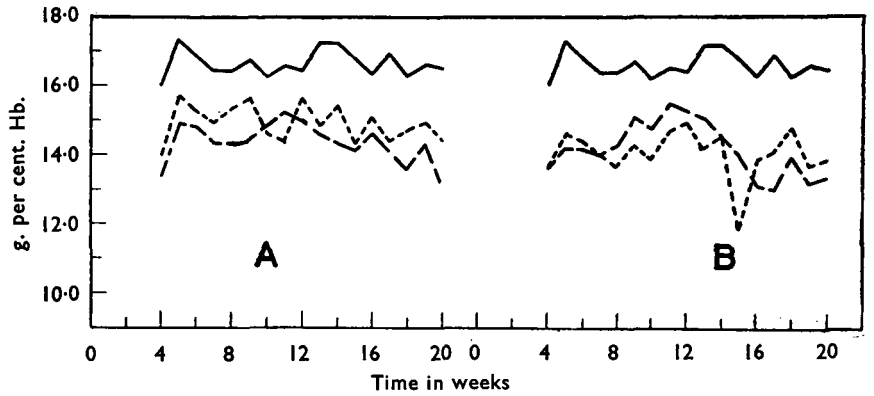

FIG. 3. Combined results of hæmoglobin determinations on both sexes of control rats and those given the two colours at different doses. A. F.D.&C. Red No. 32. B. F.D.&C. Orange No. 2.

— Control: – – – 200 mg./kg./rat, oral: — — 400 mg./kg./rat oral.

same as the controls but the body weights of the test animals were less than the controls, suggesting the possible utilization of muscle protein. In other cases the body weights of test and control animals were about the same but the organ weights differed significantly. The changes observed in the organs in these cases appeared like compensatory changes. These results do not demonstrate a correlation of organ weight and the histopathological changes except with the testes.

TABLE III

CUMULATIVE NUMBER OF DEATHS

Concentration of colour in diet	Sex	No. Rats on test	Time in weeks on test											
			2	4	8	12	16	20	24	28	32	36	40	44
FD&C Red No. 32														
Control	M	20	0	2	3	4	5	7	10	11	11	12	13	13
	F	20	0	0	2	2	3	6	7	7	7	7	7	7
0·03 per cent.	M	20	0	0	2	3	3	7	9	9	9	9	16	18
	F	20	0	0	1	1	5	5	6	6	6	6	8	9
0·75 per cent.	M	20	2	3	4	11	15	15	15	16	17	19	20	
	F	20	0	0	0	9	11	13	15	15	15	16	20	
1·5 per cent.	M	20	2	8	11	17	19	20						
	F	20	0	2	10	18	19	20						
FD&C Orange No. 2														
Control	M	20	0	2	3	4	5	7	10	11	11	12	13	13
	F	20	0	0	2	2	3	6	7	7	7	7	7	7
0·03 per cent.	M	20	0	0	1	2	4	6	6	7	8	9	13	13
	F	20	0	0	0	0	1	2	3	4	5	6	7	7
0·75 per cent.	M	20	2	5	10	17	20							
	F	20	3	6	10	17	19	20						
1·5 per cent.	M	20	3	12	17	20								
	F	20	4	10	16	20								

Hæmatology

Hæmoglobin determinations were made weekly for 20 weeks on groups of male and female rats, 10 rats to a group, given daily oral doses of 200 mg./kg. and 400 mg./kg. respectively of FD&C Red No. 32 and FD&C Orange No. 2. A slight modification of the pyridine-hæmochromogen method of Rimington was used[4]. The combined results of these determinations on both sexes are shown in Figure 3, and the mean values of the final determinations are shown in Table IV. The combined blood hæmoglobin values for both sexes show a significant decline in all groups on both colours. This trend was also evident from an examination of the data obtained on each sex.

Blood hæmoglobin values were also determined on the surviving rats from the other experiments. These values, also shown in Table IV, were about the same as those for the controls.

Histopathology

A detailed examination was made of the hæmatoxylin-eosin stained paraffin sections of a number of organs including lung, heart, liver,

TABLE IV

Comprehensive Summary of Observations on Rats fed FD&C Red No. 32, FD&C Orange No. 2 and FD&C Green No. 2

Product	Dosage	No. weeks on test	No. rats surviving / No. rats on test	Mean body weight g. ± s.e.		Mean Hg (g. per cent.) ± s.e.†	Mean organ weight, mg./g. rat ± s.e.					
				Initial	Final		Heart	Liver	Kidneys	Adrenals	Spleen	Testicles
Males												
Control		44	7/20	97·7±5·1	329·1±26·3	15·8±0·29	3·3±0·08	28·6±1·57	6·4±0·07	0·08±0·007	2·4±0·13	8·8±0·35
FD&C Red No. 32	0·03 per cent. of diet	44	2/20	98·4±6·3	330·0± 6·0	16·1±0·75	3·6±0·05	37·0±4·45	7·0±1·20	0·09±0·007	2·6±0·15	8·9±0·73
FD&C Orange No. 2	0·03 per cent. of diet	44	7/20	94·1±5·8	269·4±24·9	15·3±0·50	3·4±0·03	32·5±3·31	7·2±0·13	0·11±0·013	2·4±0·22	8·4±0·63
Control		20	4/10	118·7±5·7	251·0±11·0	17·3±0·40	3·6±0·05	34·9±1·55	7·6±0·25	0·09±0·005	2·5±0·28	9·7±0·38
FD&C Red No. 32	200 mg./kg./day	20	4/10	113·1±4·8	155·0±18·9*	14·1±0·81*	4·6±0·35*	38·2±0·95	9·5±1·55	0·15±0·038*	2·9±0·60	9·6±1·32
FD&C Red No. 32	400 mg./kg./day	20	5/10	115·7±6·4	160·2±14·0*	12·8±1·50*	4·7±0·38*	53·1±2·96*	9·6±0·71*	0·13±0·014*	4·5±0·44*	9·6±1·65
FD&C Orange No. 2	200 mg./kg./day	20	4/10	115·9±4·0	200·0±18·6*	14·1±0·40*	4·1±0·32	44·4±4·46	8·8±0·73	0·12±0·014	4·5±0·52*	11·0±0·70
FD&C Orange No. 2	400 mg./kg./day	20	4/10	110·7±4·0	152·8± 8·6*	13·8±0·64*	4·2±0·18*	44·8±1·59*	8·9±0·49	0·12±0·006	5·0±0·14*	12·9±0·47*
Control		65	8/25	45·6±2·7	323·4±20·0	17·2±0·35	3·2±0·11	30·3±1·01	6·2±0·19	0·08±0·008	2·7±0·26	8·6±0·40
FD&C Orange No. 2	0·03 per cent. of diet	65	11/25	45·5±2·7	323·8±10·9	16·6±0·19	3·4±0·07	32·6±0·82	6·1±0·12	0·08±0·004	2·6±0·11	6·5±0·38
FD&C Green No. 2	3·0 per cent. of diet	65	9/25	45·4±2·7	274·0±12·5*	16·0±0·25*	3·4±0·10	34·9±2·14	6·9±0·38	0·09±0·005	2·3±0·15	5·0±0·69*
Females												
Control		44	12/20	91·7±3·5	219·8± 4·9	15·9±0·22	4·1±0·06	35·7±0·80	7·4±0·21	0·21±0·007	3·4±0·36	
FD&C No. 32	0·03 per cent. of diet	44	11/20	91·8±2·7	214·1± 2·9	14·9±0·66	4·2±0·15	39·2±1·38*	8·1±0·27	0·23±0·010	3·9±0·64	
FD&C Orange No. 2	0·03 per cent. of diet	44	11/20	95·1±3·4	219·4± 5·8	15·6±0·22	4·2±0·13	37·8±1·36	7·8±0·18	0·24±0·011	3·6±0·15	
Control		20	9/10	93·0±3·9	166·9± 6·5	15·7±0·65	4·5±0·41	37·5±1·38	8·2±0·45	0·26±0·031	3·1±0·33	
FD&C Red No. 32	200 mg./kg./day	20	10/10	92·3±4·0	147·6± 5·8*	14·7±0·23	4·5±0·18	43·0±1·73	8·7±0·34	0·20±0·004	4·3±0·15*	
FD&C Red No. 32	400 mg./kg./day	20	7/10	91·6±3·5	124·9± 4·6*	13·4±0·45*	5·0±0·18	51·8±1·92*	9·6±0·14	0·16±0·007*	6·5±2·05	
FD&C Orange No. 2	200 mg./kg./day	20	8/10	88·8±3·3	153·6± 3·1	13·7±0·19*	4·3±0·07	40·2±1·34	7·8±0·30	0·17±0·011	5·6±0·27*	
FD&C Orange No. 2	400 mg./kg./day	20	4/10	89·9±4·0	102·8±11·2*	12·9±1·37*	5·0±0·31	55·1±5·14*	9·9±0·43	0·20±0·022	5·3±0·73*	
Control		65	15/25	39·6±2·4	227·9± 3·9	16·0±0·19	4·1±0·07	35·4±0·84	7·0±0·18	0·22±0·008	3·6±0·13	
FD&C Green No. 2	0·03 per cent. of diet	65	11/25	39·4±2·4	236·7± 6·9	16·4±0·30	3·9±0·09	31·9±1·05*	6·6±0·21	0·21±0·011	3·2±0·06*	
FD&C Green No. 2	1·5 per cent. of diet	65	11/25	39·6±2·3	213·6± 8·7	16·1±0·33	3·6±0·06*	24·4±0·39*	6·1±0·13*	0·16±0·017*	2·7±0·20*	
FD&C Green No. 2	3·0 per cent. of diet	65	11/25	39·7±2·4	210·6± 7·3*	16·0±0·21	4·3±0·12	34·9±1·16	7·3±0·17	0·19±0·005*	3·6±0·22	

* significant at $P = 0·05$ † determination on 5 rats

TABLE V

SUMMARY OF HISTOPATHOLOGICAL FINDINGS

	Control	Control	FD&C Green No. 2	FD&C Orange No. 2	FD&C Red No. 32	Control	Control	FD&C Green No. 2	FD&C Orange No. 2	FD&C Red No. 32	Control	FD&C Green No. 2	Control	FD&C Green No. 2	Control	FD&C Green No. 2	Control	FD&C Orange No. 2	FD&C Red No. 32	Control	FD&C Orange No. 2	FD&C Red No. 32	Control	FD&C Green No. 2	FD&C Red No. 32	Control	FD&C Orange No. 2	FD&C Red No. 32
Sex Dose	Male 0·03 per cent. in diet					Female 0·03 per cent. in diet					Female 1·5 per cent. in diet		Male 3·0 per cent. in diet		Female 3·0 per cent. in diet		Male 200 mg./kg./day			Female 200 mg./kg./day			Male 400 mg./kg./day			Female 400 mg./kg./day		
Number of rats on test	25	20	25	20	20	25	20	25	20	20	25	25	25	25	25	25	10	10	10	10	10	10	10	10	10	10	10	10
Number of survivors	8	7	11	7	2	15	7	12	13	11	15	11	8	9	15	11	4	4	5	9	8	10	4	4	5	9	4	7
Number of rats examined	5	5	6	7	2	4	7	6	11	10	4	6	5	8	4	6	4	4	4	6	8	10	4	4	5	6	4	6
Duration of test-weeks	65	44	65	44	44	65	44	65	44	44	65	65	65	65	65	65	20	20	20	20	20	20	20	20	20	20	20	20
Bladder parasites			3	4	1	1		2	2	3		2		3		1												
Hydronephrosis						2		1	1	1						2				1								
Chronic glomerulonephritis						1														1								
Pyelonephritis																										1		
Interstitial nephritis																										1		
Altered spermatogenesis				1										7										1	3			1
Necrotic casts in testes tubules														6														
Ovary fibroma									1																			
Ovary cystadenoma									1																			
Perioophoritis																												
Liver necrosis														1		1												
Lung papilloma																										1		
Purulent pericarditis																										1		
Laryngeal abscess																1												1
Acute tracheitis							1																					

spleen, thyroid, pancreas, stomach, small intestine, kidney, urinary bladder, adrenal, testes, ovaries and thymus. A summary of the findings is given in Table V. There were no consistent histopathological changes observed in the tissues or organs studied that could be attributed to the toxic effects of the colours with the possible exception of testicular change which was observed in animals on the higher dietary concentrations. These findings were particularly noticeable in rats on dietary levels of 3·0 per cent. FD&C Green No. 2 and in those receiving daily oral dosage of 400 mg./kg. of FD&C Red No. 32. On the 3·0 per cent. level of the green colour testicular change was a constant finding. In seven out of eight animals there was tubular atrophy and incomplete spermatogenesis. The change, although constant, was variable in degree. In some testes a few tubules showed complete absence of spermatogenic cells and in others the spermatogenic cells were greatly reduced. Several tubules in each testis contained deep-blue-staining caseous necrotic casts. Remnants of sperm were present in the necrotic debris. The spermatogenic cells and the supporting network of the tubules were undergoing varying degrees of degenerative changes. In some tubules it was apparent that the necrotic casts were being formed from the degenerating cells.

SUMMARY

1. FD&C Orange No. 2, FD&C Red No. 32 and FD&C Green No. 2 in concentrations of 0·03 per cent. in the diet did not affect growth, food consumption or food efficiency in either male or female rats.

2. In groups receiving FD&C Orange No. 2 and FD&C Red No. 32 in concentrations of 0·75 and 1·5 per cent. in the diet, there was 100 per cent. mortality before the completion of the experiment.

3. FD&C Green No. 2 in a concentration of 3·0 per cent. in the diet adversely affected the growth rate which may have been due in part to the amount of food consumed. At the 1·5 per cent. concentration in the diet of female rats no effect on growth rate, food consumption or food efficiency was observed.

4. Hæmoglobin production was not affected by 0·03 per cent. in the diet of either FD&C Orange No. 2 or FD&C Red No. 32, but oral doses of 200 and 400 mg./kg. of these two colours caused a decline in hæmoglobin values which was significant in both sexes at 20 weeks. Hæmoglobin values were not affected by any of the dosage levels of FD&C Green No. 2.

5. The only significant pathology was found in the testes of those rats receiving a dietary level of 3·0 per cent. of FD&C Green No. 2, or an oral dosage of 400 mg./kg. of FD&C Red No. 32.

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